Free Paper Presentation 4 – Basic Science including Animal Models

OL-019 Effects of HBV on signal transduction of transforming growth factor-β1 and synthesis of collagen I in human hepatic stellate cell line, LX-2 cells

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Background and Aims: Transforming growth factor-β1 (TGF-β1) pathway is key to pathological accumulation of extracellalar matrix in liver fibrosis, in which collagen type I is the main component. We aimed to observe the direct effects of HBV on signal transduction of TGF-β1 and synthesis of collagen type I in LX-2 cells.
Methods: HBV was purified by sucrose density gradient centrifugation from human serum. After LX-2 cells cultured with 3.6×10^5 copies/ml of purified HBV for 24 and 48 hours, mRNA expression of collagen type I, TGF-β1, Smad3 and Smad7 were quantified by fluorescence-quantification real-time PCR;protein expression were detected with ELISA or Western blot analysis.
Results: When LX-2 cells cultured with purified HBV for 24 hours; mRNA expression of TGF-β1, Smad3 and Smad7 were 1.8, 1.7 and 1.2 folds to control respectively; and those were 1.9, 4.3 and 0.3 folds to control for 48 hours. Protein expression of TGF-β1 in supernant did not change significantly ($P>0.05$) for 24 and 48 hours. Protein expression of Smad3 and Smad7 increased after 24 hours, Smad3 kept increasing while Smad7 show no significant differences to control after 48 hours in Western blot analysis. The mRNA expression of collagen type I were 2.2 and 1.3 folds to control after 24 and 48 hours; protein expression of collagen type I in supernant increase significantly ($P<0.05$).
Conclusions: HBV promote expression of TGF-β and its downstream moleculars, HBV increase synthesis of collagen type I in LX-2 cells.

OL-020 Circulating Toll-like receptor (TLR) 2, TLR4, and $CD4^+CD25^+CD127^{low}$/-regulatory T cells correlate with chronic hepatitis B virus infection

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Background: Toll-like receptors (TLRs) play a crucial role in early host defense by recognizing pathogen-associated molecular patterns (PAMPs) that are essential for the survival of the microorganism. $CD4^+CD25^+$ regulatory T cells (Tregs) have been shown to play an important role in maintaining peripheral tolerance against self and foreign antigens, and in suppressing T cell immune response. However, the significance of TLRs expression and Tregs in hepatitis B virus (HBV) infection has not been clearly described.
Methods: Flow cytometry was performed to assess TLR2 and TLR4 expression on monocytes and circulating $CD4^+CD25^+CD127^{low/-}$ Tregs frequency of 16 patients with acute hepatitis B (AHB), 42 patients with chronic hepatitis B (CHB), 22 asymptomatic carriers (AsC), and 20 healthy controls (NC). Influence of HBeAg and HBcAg on TLR2 and TLR4 expression was also investigated.
Results: TLR2 and TLR4 were overexpressed on $CD14^+$ monocytes in HBV-infected patients as compared with NCs. This result was confirmed by detection of a clear increase in TLR2 and TLR4 expression in HepG2.2.15 cells compared to HepG2 cells. The difference in the proportion of Tregs between NCs and CHBs was significant. A negative correlation between TLR4 expression and Tregs was found in CHBs. TLR2 and TLR4 expression decreased after HBcAg stimulation in CHBs. Upregulation of TLR2 in NCs and TLR4 in CHBs was observed following HBeAg incubation.
Conclusions: These results suggest that TLR2, TLR4, and Tregs correlate closely with HBV infection, and a potentially important interaction between innate immune responses and immunoregulation during HBV infection.

OL-021 γ-Aminobutyric acid worsen CCL4-induced rat liver fibrosis

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Objective: The mechanism underlying the liver fibrosis is still not clear. In order to clarify the effect of inhibitory transmitter in liver fibrosis, we treated rats with liver fibrosis by GABA.
Methods: In the present study, totally 18 rats were randomly divided into three groups, A group was injected by GABA+CCL4, B group was injected by CCL4, and C group was normal control. Liver fibrosis from three groups was evaluated by HE and Masson's staining. RNA was purified using the TRizol kit. The gene expression level was determined by Real-time PCR.
Results: Interestingly, degree of liver fibrosis in group A was deteriorated and emerge liver ascites. The mRNA expression level of α-SMA and TGF-βwere both up-regulated respectively 3.6 and 3.3 times compared with group C, COL I of group A was almost unchanged compared with group C but only being 10 percent of group B.
Conclusion: Our results indicated that GABA could fasten the process of rat liver fibrosis through promoting α-SMA and TGF-βexpression but not through increasing COL I synthesis.
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OL-022 Antiviral activity of notoginsenoside ST-4 against herpes simplex virus types 1 and 2

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Background: Herpes simplex viruses (HSV-1 and -2) are important pathogens for humans. HSV-1 is associated with orofacial infections and encephalitis, whereas HSV-2 usually causes genital infections. The aim of the study was to investigate the antiHSV activity of notoginsenoside ST-4 and its antiviral action.
Methods: We detected cytotoxic effect using XTT assay and antiviral activity using plaque reduction assay. And we detected the antiviral action with Virucidal assay, Attachment assay, Penetration assay and Post-infection Treatment assay. We also detected the inhibition on HSV DNA synthesis using real-time PCR assay and HSV protein synthesis tested by immunofluorescence microscopy.
Results: Notoginsenoside ST-4 was isolated from *Phyllanthus emblica*, which is a novel compound. It showed low cytotoxic with the 50% cytotoxic concentration (CC_{50}) of 1.560mg/ml and strong activity with the 50% inhibitory concentrations (IC_{50}) of 1.889μg/ml against HSV-1 and the IC_{50} of 2.758μg/ml against HSV-2. With antiviral action assays, we observed it posses obvious inhibitory effect on penetration during infections. We also detected that the inhibition on DNA synthesis and protein syn-